

Chlorophyll Removal From Edible Oils

Levente L. Diosady*

*Department of Chemical Engineering and Applied Chemistry
University of Toronto
200 College St., Toronto, Ontario, Canada M5S 3E5*

Abstract: The chlorophyll content of canola oil, especially when extracted from frost-damaged seed is high, and results in dull, dark brown oil unless very large quantities of bleaching clay are used. We found that treatment with phosphoric acid, under vacuum in the absence of moisture precipitates most of the chlorophyll. At 140°C 2400 mg/L phosphoric acid precipitated 98 % of the chlorophyll in canola oil after 15 minutes of gentle stirring under vacuum of ~ 30 mmHg (4kPa). Chlorophyll levels were reduced from 23.8 mg/L to less than 0.5 mg/L. This was further reduced to > 0.05 mg/L with caustic refining and bleaching. A similar reduction in chlorophyll level was obtained with 5000 mg/L sulfuric acid at room temperature. Mass spectrometric analysis indicates that the chlorophyll is irreversibly converted to oil insoluble pheophorbides by the treatment. A mechanism is proposed. It was shown that the precipitated pigment may be left in the crude oil, as it will be removed during caustic refining. Thus the process requires only the addition in front of the caustic refining step of a heat exchanger and a vacuum mixing vessel with 15 min. residence time. The process was demonstrated on pilot and full scales. It has been patented and commercially implemented.

Keywords: Chlorophyll removal; edible oil processing; canola oil; pheophorbides.

1. Background

Chlorophyll is a critical compound in the development and metabolism of plants, and is always present to some extent in oilseeds. During oilseed processing chlorophyll is readily extracted by hexane into the crude oil. If it is present in sufficiently large concentration, chlorophyll imparts a greenish color to the crude oil. Chlorophyll is thermally decomposed to the pigment pheophytin, which results in a dull, dark-colored oil. This pigment contributes to an off-flavor and may also promote the oxidation of the oil, thus reducing its storage stability.

While canola seeds always contain significant quantities of chlorophyll, if the seeds are damaged by early frost the chlorophyll content of the oil can increase dramatically. Since the growing season for canola is barely adequate in some of the more northern growing regions, a large percentage of the crop is always at risk of frost damage.

The problem of chlorophyll in canola oil is industry-wide in Canada. While soya oil contains typically less than 1 ppm chlorophyll, typical Canadian canola contains 13-30 ppm. The problem is much less severe in Europe,

* Corresponding author: e-mail: diosady@chem-eng.toronto.edu

Accepted for Publication: September 23, 2005

where winter rape is grown, which readily matures in that climate. The presence of high levels of chlorophyll in crude canola oil creates very serious processing problems for the refiner. Although chlorophyll and pheophytin may be removed by increasing the amount of bleaching clay used, this is expensive and not always completely effective, since the clay also adsorbs 1/3 to 3/4 of its weight in oil. Thus it is very important for the Canadian canola processing industry to develop suitable technology for dealing with the presence of chlorophyll in the seed, either by altering the extraction process to minimize chlorophyll extraction with the oil, or by the removal of chlorophyll from crude oil during the refining operations.

The scientific literature and our own research experience indicated that a number of unconventional approaches may successfully remove chlorophyll from oil. We previously investigated four completely unrelated approaches: membrane filtration, ion exchange, supercritical fluid extraction and solvent extraction [1].

There were indications in the literature that chlorophyll and its derivatives may be present in the oil not in true solution, but as a suspension of very small particles, probably colloidal in size, which would permit the removal of chlorophyll particles from the oil using microfiltration [2]. Our work indicated, that chlorophyll is present in true solution, and was not removed to any significant extent by even ultrafiltration.

While it is possible to exchange the Mg atom of chlorophyll with other cations under slightly acidic conditions in an ion-exchange reaction [3] leaving a negative charge on the rest of the molecule, reversible absorption of the chlorophyll on an ion exchange resin proved to be possible, but impractical.

Extraction of the chlorophyll with supercritical carbon dioxide was promising, but technologically too difficult at present.

Ideally the problem would be eliminated by not extracting chlorophyll into the crude oil at

all. However, a major change in the extraction process would be difficult to implement, and therefore we decided to investigate a series of chemical treatments for chlorophyll extraction that can be readily integrated into the conventional refining process.

2. Experimental techniques

2.1. Materials

Crude canola oil was obtained from Procter and Gamble Canada Inc., Hamilton ON.

All chemical reagents used were obtained from J.T. Baker Chemicals Co, Phillisburg NJ. The reagents were analytical grade. Food grade phosphoric acid (85%) was used.

Natural and acid activated bleaching clays (Vega Plus and Filtrol 105) were obtained from Pembina Mountain Clay Co., Winnipeg, Manitoba.

Chlorophyll-a and chlorophyll-b standards were bought from Aldrich Chemical Co. Milwaukee, Wisconsin.

2.2. Analytical

The concentrations of chlorophyll and other chlorophyll pigments were determined spectrophotometrically, using AOCS standard method Cc 13d-55 [4].

Free fatty acids were determined by titration in ethanol, according to the AOCS standard method Ca 5a-40 [4].

Fatty acid distribution was determined by gas chromatographic determination of fatty acid methyl esters, using the AOCS standard methods Ce 2-66 and Ce 1-62 [4].

2.3. Laboratory procedures

To test the effect of chemical addition on chlorophyll content, 300 g of degummed crude canola oil was weighed into a 500 mL Erlenmeyer, and deaerated using water vacuum at ~ 30 mm Hg (~4 kPa) for 15 min. The selected amount of reagent was added to the

deaerated oil, which was then heated under vacuum to the desired reaction temperature, with gentle mixing by a magnetic stirrer. The system was held at the reaction temperature for 10 to 60 min. then cooled to somewhat above ambient temperature under vacuum.

The oil was centrifuged at 5000 rpm for 10 min. in an IAC centrifuge with 4x500 mL fixed angle rotor, to remove the precipitated pigments. The residual chlorophyll content of the supernatant was determined. In some experiments the oil was refined and bleached without first removing the precipitate from the chemical treatment.

In selected tests the oil was caustic refined and bleached. Some 300g oil was weighed into a 500 mL flask, and a 20% excess of 3N NaOH, based on the free fatty acid content of the oil, was added with gentle mixing. The system was heated to 65°C for 15 min. and allowed to settle for 1 h. The oil was decanted, and filtered through a coarse filter paper. It was then washed twice with 5% (v/v) hot water to remove traces of soap.

Some 250 g of the refined oil was weighed into a 500 mL flask, and with gentle stirring a pre-determined amount of clay (0 to 3% w/w) was added. The slurry was rapidly heated under vacuum to 105°C, and held for 15 min. It was then allowed to cool to ~ 40°C, and filtered through a Whatman No. 1 filter paper.

3. Results and discussion

Initial tests and the literature indicated that phosphoric acid added prior to caustic refining can remove some of the chlorophyll from degummed oil.

The effects of temperature and concentrated phosphoric acid (85%) addition levels were investigated. We found that at temperatures up to 100°C the chlorophyll removal was very slow. The treatment produced a fine colloidal precipitate that was not immediately filterable. After settling for several days(!) the suspension aggregated, and could be filtered, resulting in 50-85% chlorophyll removal. At

temperatures above 100°C treatments of 10-50 min. precipitated chlorophyll, which was immediately filterable.

We systematically explored the effects of temperature, phosphoric acid addition level and residence time on the residual chlorophyll level. The results are represented in Table 1. Both increased temperature and addition level lowered the chlorophyll content. At addition levels at 2000 mg/L phosphoric acid removed more than 90% of the chlorophyll in all experiments in this series. At the highest temperature tested, 160°C, more than 96% of the chlorophyll was precipitated at addition levels above 1200 mg/L, while lower levels resulted in only small decreases in chlorophyll (Table 1).

At temperatures below 120°C the separation of the precipitated chlorophyll was difficult. This indicates that moisture present in the system interferes with the process. At higher temperatures the precipitation is complete in 15 min., and further contact does not result in any further decrease in residual chlorophyll level. Thus a treatment of 15 min. at 120°C with 2400 mg/L phosphoric acid was selected as a practical compromise, which results in 95+ % chlorophyll removal

The treated oil had a slightly increased free fatty acid level, as expected. Some of the measured acidity was due directly to the titration of the phosphoric acid, while some 0.2% was due to acid hydrolysis of the oil.

The treatment imparted a reddish hue to the oil, resulting in an increased in the Lovibond reading from 6.0R to 8.5R. This was at least partially due to the removal of the green chlorophyll, which masked some of the red tint in the crude. When crude and phosphoric acid treated oils were refined and bleached, the same final FFA and Lovibond readings were obtained with both. The untreated refined oil contained 1.7 mg/L chlorophyll, compared to <0.05 mg/L for the treated oil.

Although soybeans are typically much lower in chlorophyll than canola seeds, tests with soybean oil also gave excellent results.

Starting with chlorophyll levels of approximately 1 mg/L, the treatment resulted in chlorophyll contents of <0.05 mg/L.

The mechanism of the chlorophyll precipitation was not immediately obvious. Phosphoric acid is a strong mineral acid, but it is also a good chelating agent, and therefore we investigated the effect of a number of other acids, phosphate salts and chelating agents. At low temperature these treatments had no measurable effect. At 140°C only minor reductions in chlorophyll were observed with most of the reagents tested, only phosphoric acid had a major effect. The results are presented in Table 2.

Table 1. Effect of temperature and phosphoric acid addition level on chlorophyll removal from canola oil containing 26.8 mg/kg chlorophyll

H ₃ PO ₄ added	2400	2000	1200	1000	800
	ppm	ppm	ppm	ppm	ppm
Temperature	Chlorophyll left in oil, mg/kg				
100°C	0.4	1.9	6.7	8.7	20.3
120°C	0.6	2.0	4.9	0.3	19.4
140°C	0.3	1.0	2.5	8.6	17.0
160°C	0.2	0.5	0.8	4.5	16.9

As other acids did not remove appreciable quantities of chlorophyll, the H⁺ is clearly not the actual reagent. Curiously, phosphate salts were also ineffective in the presence of an organic acid. Typical chelating agents were also ineffective.

Sulfuric acid is a strong oxidizing agent, and darkens the oil. In high temperature treatments with concentrated sulfuric acid this made the chlorophyll measurement impossible. Niewiadomski [5] indicated that sulfuric acid oxidizes and precipitates gums, proteins

and pigments, including chlorophyll, from the oil, and therefore a range of conditions for sulfuric acid treatment were investigated. At room temperature 2000 mg/L H₂SO₄ addition resulted in significant reduction in chlorophyll. Addition levels above 4000 mg/L H₂SO₄ reduced the chlorophyll content by 98%. The treatment did not increase the Lovibond red value in the treated oil, and the refined and bleached oil had a lower red value than the untreated oil.

Mass spectrometric analysis [6] of the oil samples and of chlorophyll standards dissolved in pure octane showed that the phosphoric acid treatment converted the chlorophyll to oil-insoluble pheophorbides (Figure 1).

We believe that reaction proceeds in the absence of water only, by protonation of the carbonyl group, followed by de-alkylation. This releases the non-polar aliphatic side chain, leaving the porphyrin structure with a carboxylic acid side-chain, which is oil insoluble.

The proposed mechanism predicts that as the side-chain is completely removed, the precipitation of the chlorophyll is irreversible: the removal of the phosphoric acid by neutralization, or the addition of moisture would not re-solubilize the chlorophyll, or the precipitated pheophorbide into the oil. This lead to integrating the acid treatment into the refining process. Phosphoric acid was added to the crude oil at 140°C under vacuum with gentle mixing. After 15 min., the oil was cooled and caustic refined, without first removing the precipitated chlorophyll breakdown products. The precipitate was removed with the soapstock, and the oil was bleached.

The phosphoric acid treatment readily fits into the normal refining practice, as it uses a chemical that is already added to canola oil during or after degumming. The process would require the addition of a heated vacuum mixing vessel and a centrifuge. Sulfuric acid treatment, while it is simpler in some ways, requires more acid, and requires the

introduction of another chemical into the process, which has some potential labeling and safety implications. The process block diagram is presented in Figure 2.

Comparison of the chlorophyll levels after refining and after bleaching indicated no significant differences in chlorophyll content between samples that had the precipitate removed prior to refining, and those that eliminated that separation step. After bleaching both of treated oils contained <0.05 mg/L chlorophyll.

The reaction mechanism indicates that the chlorophyll may be removed at any stage in the refining process, where the chlorophyll is still intact, and the process allows the oil to be heated to a high temperature, in the absence of water. Thus the phosphoric acid treatment may be introduced prior to either acid degumming or bleaching for physically refining plants, since both of these process stages are followed by a filtration or centrifugal separation step. We tested this hypothesis on a large pilot-plant scale. The results are presented in Table 3.

Table 2. Effect of chemical treatments on chlorophyll removal

Reagent	Residual chlorophyll ppm	Chlorophyll reduction %
Initial chlorophyll content	23.8	0
Citric acid (aq)	22.09	7.2
Potassium dihydrogen phosphate (aq)	22.48	5.5
Hydrochloric acid	19.76	17.0
Sulfuric acid*	n/d	n/d
Maleic anhydride (aq)	21.59	9.3
Sodium EDTA	22.43	5.8
Acetic acid	22.32	6.3
Sodium sulfate + acetic acid	22.07	7.3
EDTA + acetic acid	22.32	6.3
Potassium dihydrogen phosphate + acetic acid	22.41	5.9
Malic Acid	22.5	4.3
Monoglyceride citrate	22.5	4.3
Phosphoric acid	0.30	98.7
Conditions: Temperature 140°C Addition level 2000 ppm Contact time 10 minutes All figures are averages of three replicate treatments with a relative standard deviation of less than 10%.		

* Not measurable, due to the dark color of the oil.

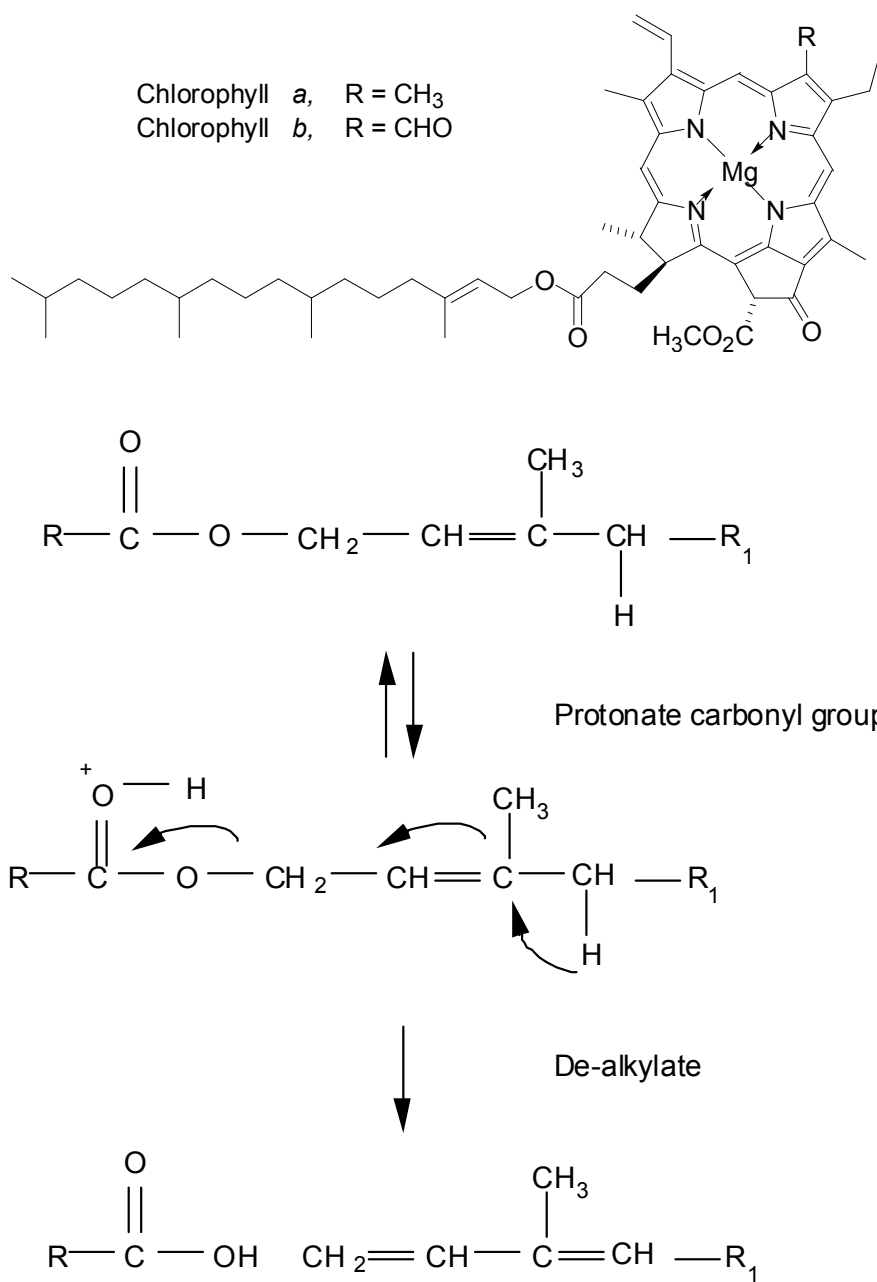


Figure 1. Mechanism of chlorophyll removal

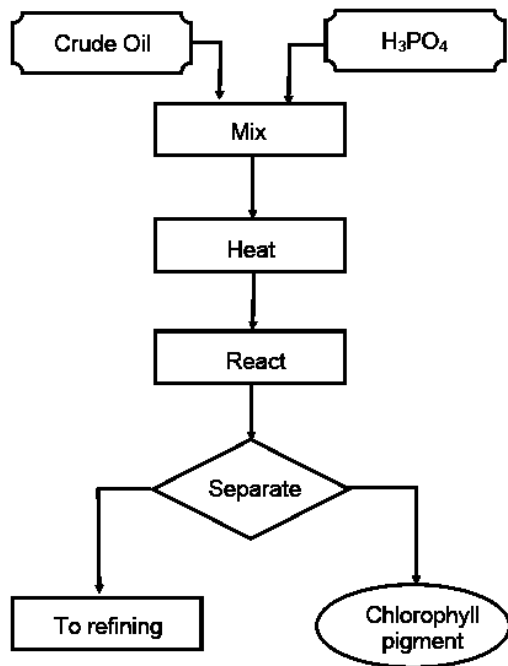


Figure 2. Flow diagram for pre-refining chlorophyll removal

Table 3. Effect of phosphoric acid treatment, with and without the removal of the precipitated pheophorbides prior to caustic refining

Material	Without treatment	With treatment	
		With	Without
		separation	
	Chlorophyll content		
	ppm	ppm	ppm
Original oil	23.2	23.2	23.2
Phosphoric acid treated	n/a	2.7	n/a
Caustic refined	18.2	0.8	0.5
Bleached	1.7	<0.05	<0.05

The elimination of the precipitate separation step makes the process readily adaptable to typical refineries. The addition of a heat exchanger that heats the oil to 140°C and a

vacuum mixing vessel with 15 min. residence time are the only modifications required. The block flow diagram is presented in Figure 3. Pilot scale tests have confirmed the laboratory results, and after a full-scale test in Procter and Gamble Inc.'s Hamilton plant, the process was patented [7], and successfully implemented in a full scale caustic refining plant.

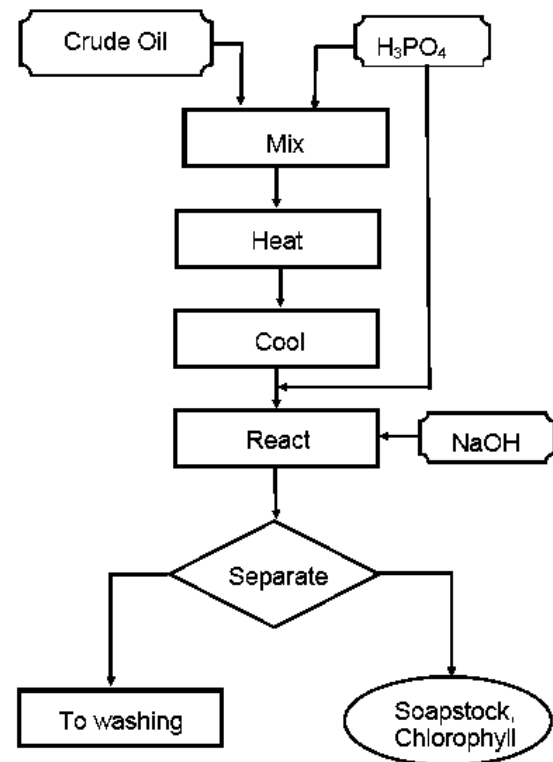


Figure 3. Flow diagram for integrated chlorophyll removal

Acknowledgements

The author would like to thank Joseph Alberti, Ahmed Hussein and Chris Beharry for their technical assistance, and Professor D.G.B. Boocock for his theoretical insight. The work was financially supported by NSERC, the URIF program of the Government of Ontario, and Procter and Gamble Inc.

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